

Amersham™ ECL™ Gel System Compatibility Guide

This guide provides useful hints and tips on how to use Amersham ECL Gel system for SDS-PAGE. It contains illustrative examples of system performance, optimized Western blotting transfer protocols, as well as trouble shooting guidelines to help you achieve optimal results.



Compatibility Guide

Electrophoresis applications	Recommendations
Protein SDS-PAGE	Amersham ECL Gel is available in 10, 15 and 2 well formats, and as either homogeneous (10 and 12%), or gradient (4-12%, 8-16%, and 4-20%) gels. Homogenous and 8-16% gradient gels have a 4% stacking gel. Gel buffer is Tris-HCL pH 6.3. %C=2.6% (w/w); %T=Total concentration (w/v) of acrylamide+ bisacrylamide is given as percentage in the respective Amersham ECL Gel product name. Running buffer: Amersham ECL Gel Running Buffer; 25 mM Tris, 192 mM Glycine, 0.1% SDS, pH 8.3.
Protein Native PAGE	Running buffer: 25 mM Tris, 192 mM glycine, pH 8.3. See figure 16 for example result.
DNA/RNA PAGE	Running buffer: 25 mM Tris, 192 mM glycine, pH 8.3.

Preparing Amersham ECL Gel Box	Recommendations
Power supply	Recommended power supply is EPS 301. See ECL Gel Box User Manual (28-9943-33) for more information on power supply compatibility.
Running buffer	Running buffer for SDS-PAGE: Amersham ECL Gel Running buffer ; 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3. Note: It is important to follow buffer volume recommendations; 90 ml running buffer in each buffer chamber (Figure 1), and 6 ml running buffer in the sample well chamber (Figure 2).



Figure 1. Add 90 ml of Tris-Glycine-SDS running buffer to each buffer container.



Figure 2. Add 6 ml of Tris-Glycine-SDS running buffer to the sample well container.

Note: Use of non-Tris Glycine based buffers such as MES or MOPS buffer for Tris-HCL based Amersham ECL Gels will have a negative impact on results (Figure 3).

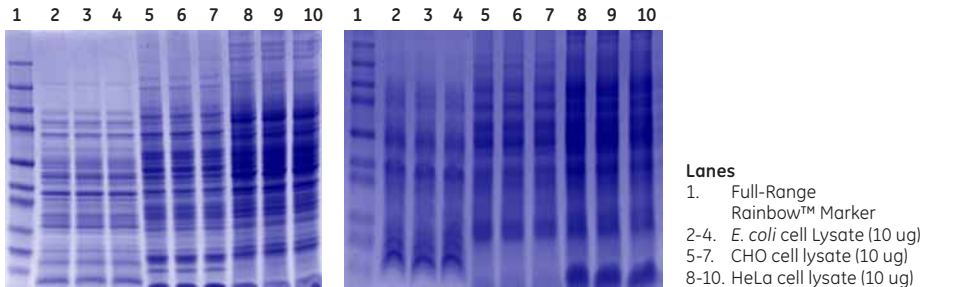


Figure 3. *E. coli*, CHO cell, and HeLa lysates separated on Amersham ECL Gel, 4-20%, using Amersham ECL Gel running buffer (Tris-Glycine-SDS) (a) and NuPAGE® MES SDS buffer (Invitrogen) (b). MES buffer is suitable for Bis-Tris gels only, and has a negative impact on separation using Tris-HCL based Amersham ECL Gel.

Running temperature	Amersham ECL Gel should be stored at +2 to +8°C. Amersham ECL Gel Running Buffer can be stored and used at room temperature (+15 to +25 °C), (Figure 4). Note Remove gel from refrigerator just prior use. For optimal resolution, gels should be cold when run in Amersham ECL Gel system.
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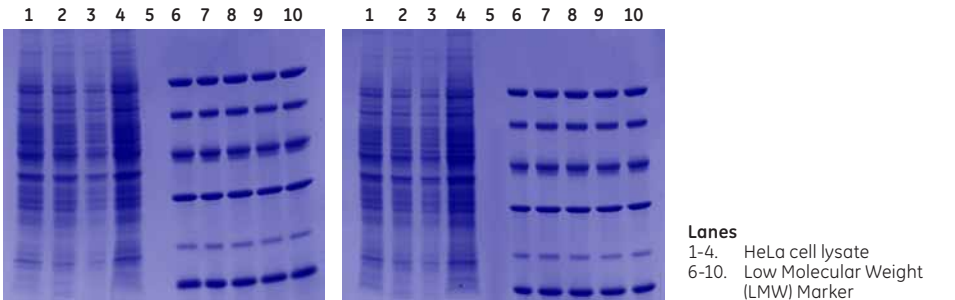
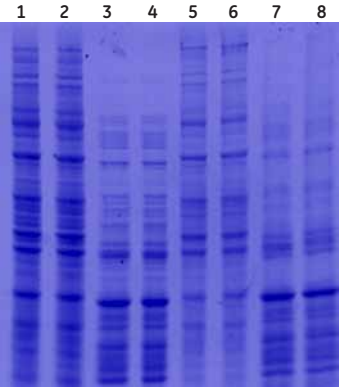
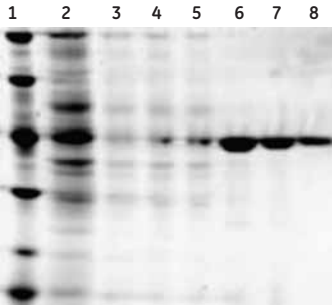
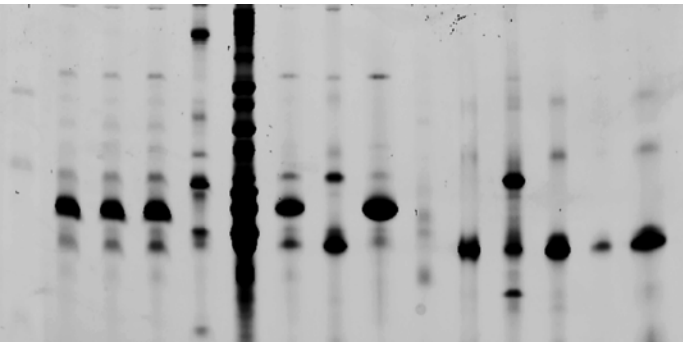


Figure 4. Separation on Amersham ECL Gel, 4-20% using cold (left) and room tempered (right) running buffer. The temperature of the running buffer does not affect the separation.

Sample loading	
Sample loading buffer	Tris-HCL-SDS or other buffer suitable for the application.  Lanes 1-2. CHO cell Lysate, Tris-HCL-SDS sample loading buffer 3-4. <i>E. coli</i> lysate, Tris-HCL-SDS sample loading buffer 5-6. CHO cell lysate, Novex Tricine Sample buffer 7-8. <i>E. coli</i> lysate, Novex Tricine Sample buffer
Figure 5. CHO cell, <i>E. coli</i>, and HeLa cell lysates (10 µg total protein) separated on Amersham ECL Gel, 10%, using Tris-HCL-SDS sample loading buffer, and Novex® Tricine SDS Sample Buffer (Invitrogen). The results demonstrate that protein in Tricine based sample loading buffer may result in slightly affected resolution of protein bands compared to proteins in Tris-HCL based SLB.	
Salt concentration	High salt concentration in sample may result in vertical streaking of bands. Use gel filtration (e.g. Vivaspin Sample Concentrators) for buffer exchange if needed. Note: In general, concentration of imidazole up to 20 mM does not affect protein separation.  Lane 1. Low molecular weight (LMW) markers 2. Start material, MBP-(His)₆ in <i>E. coli</i> lysate 3. Flow through, 5 mM imidazole 4. Flow through, 10 mM imidazole 5. Flow through, 20 mM imidazole 6. Eluted sample, 5 mM imidazole during binding 7. Eluted sample, 10 mM imidazole during binding 8. Eluted sample, 20 mM imidazole during binding
Figure 6. Samples from flow through and elution fractions containing different concentrations of imidazole are separated on Amersham ECL gels. The result shows that concentrations of imidazole up to 20 mM do not affect the protein separation or cause vertical streaking.	
Sample well volume	10 wells; 35 µl, 15 wells; 20 µl, 2 wells; 100 µl.
Protein amount	Maximum of 0.5 µg protein/band per well can be loaded. Overloading may cause smearing and distortion (Figure 7). 
Figure 7. Example of band broadening and smearing due to overloading. Plasma fractions in an IgG purification process, stained with Deep Purple™ Total Protein Stain.	
Pipette tips	Use regular pipette tips for sample loading. Flexible gel loading tips should not be used. Note: Be careful not to thrust the pipette tip down into the well when loading. This may cause the well bottom to break and leak.
Recommendations	Use a prestained marker, e.g. Rainbow Molecular Weight markers, to monitor separation, and confirm transfer from gel to membrane.
Empty wells	Load an equal volume of sample loading buffer into any unused well to ensure uniform mobility.

Staining methods

Recommendations

Coomassie

Amersham ECL Gel works well with standard Coomassie® staining protocols (Figure 8). See also recommendations in ECL Gel Box User Manual, (28-9943-33).

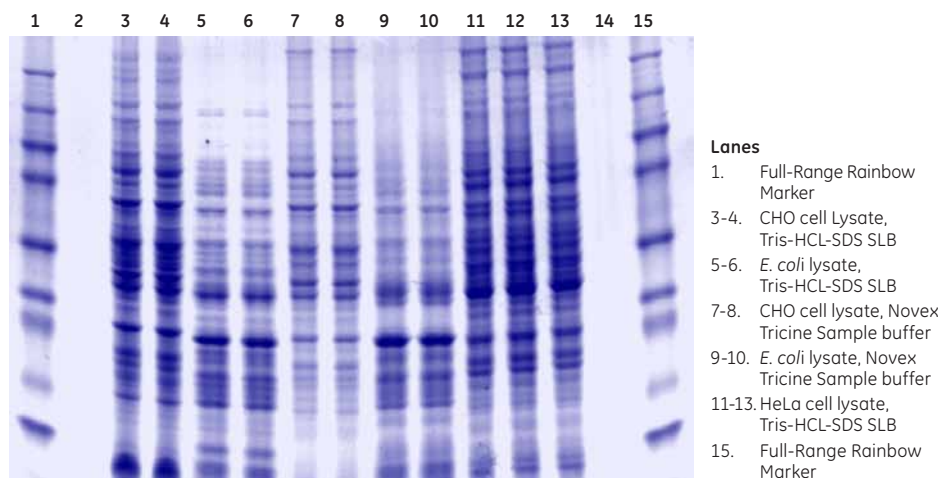


Figure 8. Staining of Amersham ECL Gel, 4-20%, using SimplyBlue™ Safestain (Invitrogen).

Silver stain

Amersham ECL Gel works well with PlusOne™ Silver Staining Kit, Protein (Figure 9). Follow recommendations in ECL Gel Box User Manual (28-9943-33).



Figure 9. Staining of Amersham ECL Gel, 4-20%, using PlusOne™ Silver Staining Kit, Protein. Dilution series from 15.6-0.03 ng protein in 50 kDa band.

Deep Purple™ Stain

Amersham ECL Gel works well with Deep Purple Total Protein Stain, RPN6305 (Figure 10). Follow recommendations in ECL Gel Box User Manual (28-9943-33).

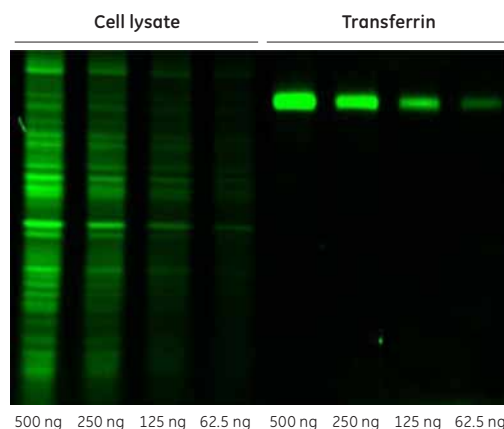


Figure 10. Staining of complex and purified samples after SDS-PAGE on Amersham ECL Gel, 4-20%, using Deep Purple Total Protein Stain.

Western blotting transfer protocols

Tank transfer

Recommended running conditions using TE 22 Tank transfer unit

Parameter	Value
Voltage	Constant voltage: 25 V Current (A) and power (W): Non-limited Note: Voltage may be adjusted depending on protein size and characteristics.
Temperature	4°C Note: Perform the transfer in a cold room. Use a magnetic stirrer to ensure buffer circulation.
Time	2.5 h Note: Time may be adjusted depending on protein size and characteristics.

- Use pre-chilled transfer buffer**
Tris-glycine buffer: 25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3. Prepare fresh, and pre-chill to reduce heating.
Hints & Tips: Lower methanol concentration (10%), and addition of 0.1% SDS may improve transfer of large proteins.
- Equilibrate the gel**
Equilibrate the gel in cold transfer buffer for >20 minutes.
- Follow step-by-step protocol for tank transfer outlined in Amersham ECL Gel Box User Manual (28-9943-33).

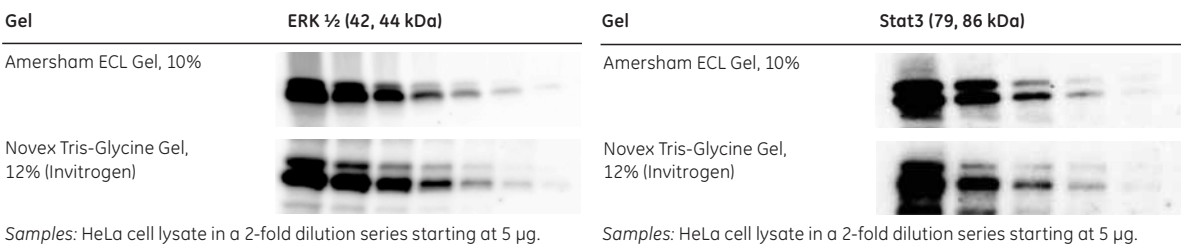


Figure 11. Tank transfer with subsequent Western blotting detection of ERK1/2 (left) and Stat3 (right). Comparison shows similar performance between Amersham ECL Gel, 10%, and Novex Tris-Glycine Gel, 12% (Invitrogen). Gel electrophoresis and transfer was performed in triplicates according to the manufacturer’s instructions.

Semidry transfer

Recommended running conditions using TE 77 semidry transfer unit

Parameter	Value
Current	0,8 mA/cm²
Time	80 minutes Note: Recommended transfer time is for mid-size proteins. The time may be longer for larger proteins (90min), and shorter for small proteins (60min).

- Transfer buffer**
Tris-glycine buffer: 25 mM Tris, 192 mM glycine, 20% methanol, 0.1% SDS, pH 8.3. Prepare fresh.
Hints & Tips: A discontinuous buffer system may improve transfer efficiency. Use 25 mM Tris, 192 mM glycine, 20% methanol in anode buffer, and 25 mM Tris, 192 mM glycine 0.1% SDS in cathode buffer.
- Equilibrate the gel**
Equilibrate the gel in cold transfer buffer for 20 minutes.
Note: Equilibration in transfer buffer removes SDS from the gel This preserves the shape of the gel, and prevents the gel from sticking to the membrane.
- Follow step-by-step protocol outlined in TE 77 User Manual (28-4025-91).

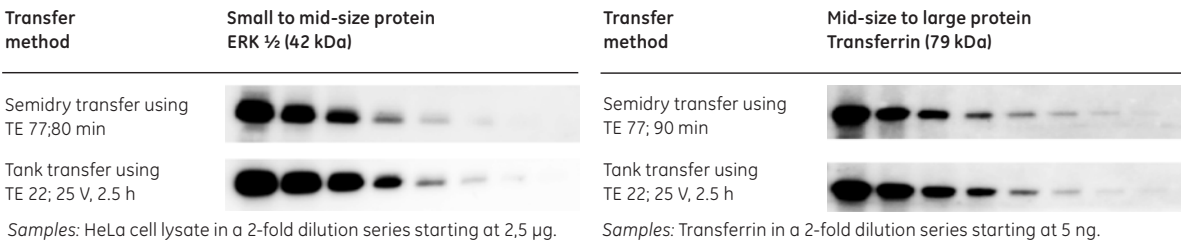


Figure 12. A comparison of semidry and tank transfer using the Amersham ECL gels shows similar results in Western blotting detection of ERK1/2 (left) and transferrin (right).

Typical results

In this section, typical appearance of Amersham ECL Gel, before, during, and after electrophoresis is illustrated. Examples from protein separation under native and denatured conditions respectively are provided, as well as an example of DNA separation.

Removing the comb: Excess gel pieces

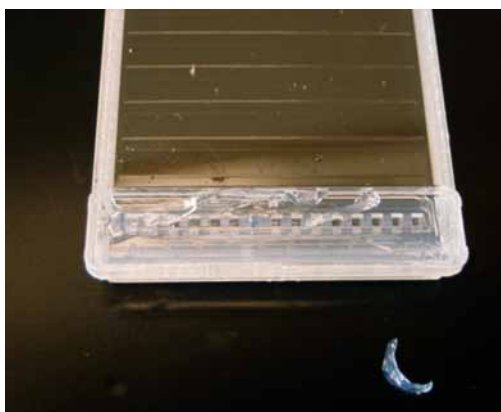


Figure 14. As the comb is removed, excess gel pieces can be detached from the well container. This will not affect gel performance, but pieces should be removed to facilitate sample loading.

Performing the separation: Appearance of front during run

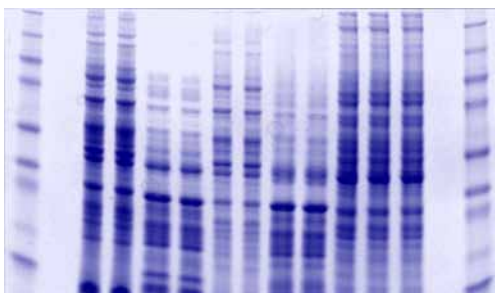
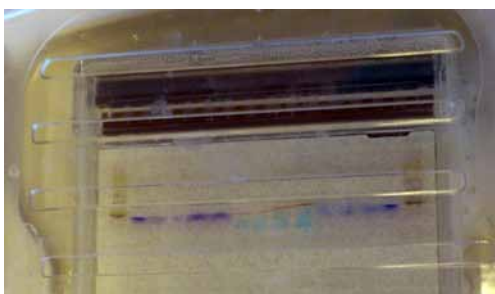


Figure 15. As the gel is run in Amersham ECL Gel box, the front may appear less straight than expected (upper). This typically has no effect on resulting separation after completion of the electrophoresis run (lower).

Native conditions: Blue Native PAGE using Amersham ECL Gel

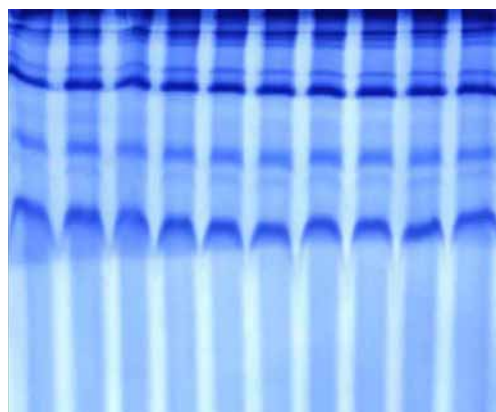


Figure 16. Blue Native data kindly provided by Prof. Dr. H.P. Braun, Institute of Plant Genetics, Leibniz University Hannover.

- Ready-made gel: Amersham ECL gel 4-12%, 10 wells
- Cathode buffer: 50 mM Tricine, 15 mM Bis-Tris, 0.02% w/v Coomassie G-250 (90 ml/run)
- Anode buffer: 50 mM Tricine, pH 7.0 (90 ml/run)
- Thylakoid membrane protein complexes of *Arabidopsis thaliana* were solubilized with 5 % (w/v) digitonin in solubilization buffer (150 mM K-Acetate, 30 mM HEPES, 10 % v/v glycerol, pH 7.4). Detergent:chlorophyll ratio was 50:1. Unsolubilized material was removed by centrifugation at 18000 g for 10 min. Each well was loaded with solubilized membrane protein complexes corresponding to 10 µg chlorophyll. Prior loading 1 µl of 5 % (w/v) Coomassie G-250 in 750 mM amino-caproic acid was added to each sample and the sample volume was adjusted to 21 µl using solubilization buffer without digitonin. All steps were carried out on ice/at 4 °C.
- Running conditions: Pre-run 12 min at 160 V prior to sample loading, then 120 min at 160 V at room temperature
- Poststain: Fixation solution [40% (v/v) Methanol, 10% (v/v) acetic acid in ddwater] over night. Visualization of proteins colloidal Coomassie staining (Neuhoff 1988)

acc. Wittig, I., Braun, H.P. and Schagger, H. (2006): Blue-Native PAGE. NATURE Protocols 1, 418-428.

Nucleic acid separation on Amersham ECL Gel

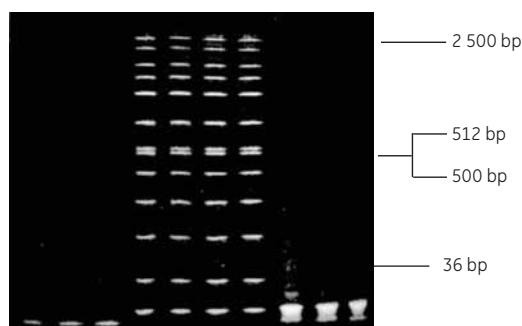


Figure 17. Example of DNA separation on Amersham ECL Gel, 4-12%, run at native conditions with Tris-Glycine buffer.

Trouble shooting

Amersham ECL Gel Box

Symptom	Possible cause	Remedies
Low or no current during run	Incomplete circuit	• Add 90 ml running buffer to each tank, and 6 ml running buffer in well chamber • Ensure that the tape is removed from the gel cassette • Ensure that the lid is in place
No green light, low current (<20 mA) at start	Incomplete circuit	• Check all power connections • Check buffer level in tanks • Ensure that the gel cassette is in place • Ensure that the tape is removed from the gel cassette
Green light, but only approx 35 mA at start	Leakage between tanks, with or without gel cassette	• Check that the tanks are not overfilled • Check if the unit is damaged and needs to be replaced
Green light, but approx 65 mA at start	Leakage between tanks, with gel cassette	• Check that the tanks are not overfilled • Check if the unit is damaged and needs to be replaced

Protein separation

Symptom	Possible cause	Remedies
Vertical streaking of bands	• Sample overload, see Figure 4 • Poorly soluble or weakly charged particles (such as carbohydrates) in sample • High salt concentration in sample • Contaminants such as cell membrane or DNA complexes in sample	• Load maximum of 0.5 µg protein/band per well • Centrifuge the amples before loading • Change pH of sample buffer • Heat sample together with SDS • Decrease salt concentration using gel filtration
Poor separation	• Incorrect ECL Gel type selected • Separation time not sufficient	• Use an ECL Gel type that separates in the desired molecular weight range • Increase the electrophoresis run time
Bands spreading horizontally across gel	• Sample overload	• Load maximum of 0.5 µg protein/band per well
Smiling effects across gel	• Uneven heating of gel	• Use cold gels and pre-chilled running buffer to reduce heating effects • Run gels at lower voltage for longer time; e.g. 125 V for 90 minutes

Western blotting transfer

Symptom	Possible cause	Remedies
No or little protein transfer to membrane	• Too short transfer time/ low voltage; Proteins left in gel • Too long transfer time/high voltage; Proteins passed through the membrane	• Optimize transfer conditions, starting from recommended transfer conditions outlined in this guide
Gel sticking to membrane after transfer	• Uninsufficient equilibration of gel	• Equilibrate gel in transfer buffer >20 min before transfer step

Ordering information

Product	Product code		
	2 wells Pack of 10 gels	10 wells Pack of 10 or 2 gels	15 wells Pack of 10 gels
Amersham ECL Gel 10%	28-9901-60	28-9898-04 28-9898-08*	28-9901-55
Amersham ECL Gel 12%	28-9901-61	28-9898-05 28-9898-09*	28-9901-56
Amersham ECL Gel 4-12%	28-9901-62	28-9898-06 28-9901-51*	28-9901-57
Amersham ECL Gel 8-16%	28-9901-63	28-9898-07 28-9901-52*	28-9901-58
Amersham ECL Gel 4-20%	28-9901-64	28-9901-54 28-9901-53*	28-9901-59
Amersham ECL Gel Box			28-9906-08
Amersham ECL Gel Running Buffer			29-9902-52
PlusOne Silver Staining Kit, Protein			17-1150-01
Full-Range Rainbow Molecular Weight marker			RPN800E
EPS 301			18-1130-01

* 2 gel pack

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifescience.com/eclgel

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